

Leukemia and the Somatic Risks of Chemical Mutagens

by E. B. Lewis*

The chief biological risks associated with exposure to chemical mutagens are likely to be the induction of mutations in germ cells and of cancers in somatic cells. This workshop has been concerned primarily with developing test systems for assessing the germinal risks. Since the induction of cancers may represent a burden to society and the individual which is comparable to, and possibly much greater on a short-term basis, than the induction of new hereditary defects, it is important to supplement genetic test systems with somatic ones. Moreover, there would appear to be an increasing need to devise somatic test systems which would have the capability of early detection of a sudden rise in cancer risks in human populations.

Since for most cancers in adults the interval between induction and onset of symptoms is probably many years it would clearly be inefficient to use such cancers in an early-warning system. A notable exception, however, is leukemia, the acute form of which may have a minimal latent period of no longer than two years, judging by a follow-up study (1) of adults exposed to ionizing radiation, and it is possible that the minimal latent period is as short as one or two months. If the association of an elevated risk of childhood leukemia with prenatal irradiation (2,3) is a causal one, then the minimal latent period in this case

can be shown to be no longer than three years, in the sense that the elevated risk has already begun to be manifested in two-year old children (4-6).

I would like to propose that serious consideration be given to initiating a program of prompt monitoring and analysis of leukemia deaths on a national scale. I will outline only a few of the reasons for thinking that such a program is feasible and potentially able to provide early detection of any increase in somatic risks that might result from environmental agents. At the outset, it should be emphasized that the proposed program will be quite restricted in the type of somatic risk it can hope to detect. Thus, a chemical agent that is able to reach and induce cancers in such sites as the skin, lungs, or lining of the digestive tract may fail for one reason or another to reach either the bone marrow or the germ cells and therefore be neither leukemogenic nor mutagenic. On the other hand, it seems likely that chemical agents which are able to reach the bone marrow and to induce leukemia will tend to reach all organs and therefore be general carcinogens and often mutagens as well.

The principal reasons for singling out leukemia as the disease of choice for a program of early risk detection are: (a) the acute form of the disease may, as already noted, have an extremely short latent period; (b) leukemia tends to be more accurately diagnosed and more accurately reported on death records than are other can-

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cers (7); (c) age-adjusted leukemia death rates in the U.S. white population appear to have largely stabilized, judging by the experience in the years 1959–1967 inclusive, which is the most recent period for which data are available (8); and (d) there is little or no evidence of seasonal variation in the incidence of the disease in the U.S. (9) or of clustering of cases (10).

Leukemia death rates would need to be monitored on a national as opposed to a regional basis, not only to have enough numbers to examine trends by type of leukemia, age, race, and sex but also because the population at risk is easier to estimate for the U.S. as a whole than it is for regions which tend to have ill-defined patterns of migration. It would, of course, be much more desirable to monitor leukemia incidence rates (number of new cases arising in a given time interval) rather than death rates. However, until national registries of cancer cases in this country are developed, it will be necessary to rely upon death records. Moreover, advantage can be taken of the circumstance that for acute leukemia, in adults especially, there is a relatively short period between time of clinical onset and death; for example, the median survival time for adults over 45 was only increased from one month to two months in the period from 1940 to 1962 (11). Hence, for acute leukemia in older adults, death rates are expected to parallel incidence rates quite closely, at least until better survivorship is achieved.

I would like to cite an example which illustrates the power of death record studies to identify associations between leukemia mortality and environmental factors. An association between farming and leukemia in this country was first detected by Guralnick (12), who analyzed cause of death in relation to occupation among U.S. males (age 20 to 64) for the year 1950. She observed a mortality rate from leukemia which was significantly higher in white agricultural workers than it was in the general male population even though mortality rates from all categories of cancers combined were

significantly lower in the agricultural group. Unfortunately, studies of cause of death in relation to occupation have not been carried out at a national level in the years since 1950. However, death record studies made in the State of California for the years 1959–1961 showed a similar association between farm residence and leukemia mortality (13). More recently, Milham (14) not only confirmed the association with farm occupation in a study of death records for the States of Washington (for the years 1950–1967) and Oregon (1950–1964) but had sufficient numbers to analyze the association in relation to six types of farming. By a matched control technique he found that the leukemia risk was elevated in each of the six types of farming with the highest risk occurring in poultry farmers.

A specificity of association to type of leukemia was also noted by Milham (14), who found that lymphatic leukemia tended to be elevated proportionately more than the myeloid type when farm workers were compared to a matched control group. By contrast, in persons exposed to ionizing radiation the myeloid types are elevated proportionately more than the lymphatic ones; indeed, chronic lymphatic leukemia appears not to be readily inducible by ionizing radiation if it is inducible at all (1). Milham (14) suggests that an animal tumor virus may be the agent responsible for the increased risk of leukemia in farm workers.

For our purposes there are a number of points of interest in this example of an association between occupation and leukemia mortality. (a) The original association was identified solely by the use of death record data correlated with census estimates of the number of farm workers. (b) Only a national study can yield enough cases of leukemia within the space of one year or less to detect the rather weak associations which are present when leukemia mortality is related to all types of farming combined. (c) The existence of an association that is specific to a certain type or types of leukemia can be expected to increase greatly the re-

solving power of an early risk detection program. (d) Although death record studies can identify a risk, further epidemiological studies will generally be needed to identify the nature of the factor(s) involved.

A program of prompt monitoring and analysis of leukemia deaths would require careful planning and the cooperation of a number of agencies. One might envisage the initial goal to be the detection of any significant changes in death rates within one or two years after those changes had occurred. The ultimate goal might be to reduce this time interval to a few months. Death records with mention of leukemia would be singled out for special processing. Such a selection procedure would avoid waiting until all death records had been coded to underlying cause. Coding to cause by the rules of the Eighth International Revision, adopted in 1968, should insure adequate breakdown of leukemia deaths by type. Prior to 1968, deaths ascribed to acute lymphatic or acute myeloid leukemia, two of the most useful categories for present purposes, were "lost" to analysis simply as the result of an artifact of grouping imposed by the coding rules then in effect. It would be highly desirable to code from the records data on a number of relevant items which are not regularly coded in the case of all records; namely, such items as, for example, occupation and industry, interval between onset and death, and whether residence is on a farm.

To give some idea of the number of records that would be available for analysis, there were 14,375 deaths certified to leukemia in the U.S. in the year 1968, the most recent year for which data have been published (15). Under acute leukemia there were 7494 deaths, the bulk of which fell into three categories: 1842 acute lymphatic; 3126 acute myeloid; and 1847 acute deaths without mention of cell type.

It would be envisaged that the basic data would be analyzed for time trends in age-specific death rates for each major type of leukemia. Burbank (8) has analyzed such trends for leukemia (and other cancers) in

the U.S. for the nine-year period, 1959-1967. He has paid particular attention to the problem of determining the statistical significance of observed trends and his procedures are model ones for future investigations.

If occupation is coded from the records, a single year should provide sufficient data, judging by the 1950 study (12) already cited, to determine the present status of the association of farming with leukemia. The primary purpose of the proposed monitoring program would still be to detect any sudden changes in leukemia mortality rates that might be due to environmental agents. Such a program would also have the resolving power of detecting annual trends in already recognized associations as well as identifying new associations that may already have existed but have gone unrecognized because of lack of sufficient data.

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Discussion

Dr. C. Langley (*Univ. of Wisconsin*): How many state Health Departments keep monthly records on leukemia?

Dr. Lewis: All states keep monthly death records.

Dr. H. E. Sutton (*Univ. of Texas at Austin*): This discussion on leukemia brings up a point which you alluded to, that is the enormously increased risk from early embryonic radiation. I'm not sure what the current risk figure is; in any event it raises the possibility that perhaps we are most susceptible to mutation before our repair systems are fully developed and perhaps the additional mutation after birth is after all rather trivial. I think this is more pertinent to leukemia. With most test systems that are used, one starts with an adult male and subjects him to radiation or other treatment; perhaps if one used the experimentally more complicated method of going back and treating him while he's still an embryo we might find that in fact the mutational risk is enormously higher.

Dr. Lewis: You're quite right that there is a relatively high risk but only for 10 years. There is a curious development first pointed out some years ago by Doll that older persons become relatively sensitive to radiation, as the fetus does. What could easily be involved is the phenomenon that initially there are relatively few cells that could be inducible as far as leukemia is concerned, but with aging, because of the breakdown of systems of various kinds the effective number of cells susceptible to induction increases exponentially.

Dr. Sutton: I saw recently a report that among young children where leukemia is induced there appeared to be two populations, one sensitive and one resistant.

Dr. Lewis: There are studies by Gibson and others in which it was noted that pathological factors were interacting with radiological factors. I don't think it has yet been proven that you have to have an association of radiation and another specific variable.

Dr. Sutton: Couldn't one of the other variables be the genetic background in the person?

Dr. Lewis: Oh, by all means it could be. I guess you're thinking of Fanconi's anemia.

Dr. Sutton: No, but that's a nice example.

Dr. R. F. Kimball (*ORNL*): To return to Dr. Sutton's remarks that repair systems, by that I'm taking it to mean DNA repair, might be ineffective in early embryos. I don't know if there is such evidence. Immunological systems are something else again, but I was not aware that there was any evidence at all that DNA repair systems had any kind of periods in the development.

Dr. Sutton: I don't know of any evidence, but there are many other enzymes that simply do not become fully functional on day one.

Dr. R. L. Capizzi (*Yale Univ.*): A comment and a question. Perhaps chronic lymphatic leukemia (CLL) ought not to be dismissed, because it might be a useful disease to follow. The division into B cells and T cells, i.e., bone marrow or bursa-derived and thymus-derived lymphocytes, is a useful way of dividing the CLL population. There have been some patients who exhibit pure B cell leukemias and some that exhibit pure T cell leukemia. The other interesting feature about this disease is that some of the CLL patients will secrete specific immunoglobulins, the genetics of which have been worked out nicely and which may indicate a specific clonal origin of the leukemia. The only problem in an epidemiological sense might be that, since this disease appears in an older population, we may be dealing with a very long latency period. However, a good epidemiologist has lots of questions and lots of ways of evoking answers and recall in the patients that are interviewed. I think it might be very useful disease to include.

The question is: Lots of studies have been done on leukemia clusters, in particular those sponsored by the CBC in Atlanta; infectious, chemical, or radiation exposures in a community or family were investigated. Do you have any comments on that?

Dr. Lewis: First on the CLL: it seems to me this reinforces the point I was trying to make, that you need that as a control group, even if it isn't radiation-induced. I do want to stress that you wouldn't restrict yourself to the radiation type you would want to include that very type. I gather originally it was thought that CLL's were nonclonal and now all of them are turning out to be clonal.

Dr. Capizzi: The methodology is being developed for the recognition.

Dr. Lewis: With respect to your question about the clusters, what I've read on this is that it always seems as if the most careful epidemiological investigation indicates that it is not a true cluster but a statistical cluster, i.e., one that you would expect on a chance basis.

Dr. Capizzi: There are two other diseases that may be interesting in this category. One is paroxysmal nocturnal hemoglobinuria (the Machiafabba-Michelli syndrome), which appears in many patients

after exposure. Patients excrete hemoglobin in their urine in an intermittent fashion, in paroxysms, during the night. This disease frequently is recognized after exposure to a chemical agent—most of the agents that will suppress bone marrow function—like benzene or organic solvents. Some of these patients will then go into an aplastic phase and some of them will then also develop a myelogenous leukemia, perhaps related to the initial exposure. As far as I know, there is not a lot of epidemiological study on this disease. The other disease which terminates frequently in leukemia is polycythemia vera, and we don't know what causes that. Initially it is an increase in red cells, but there is an increase also in the white cells and platelets. Patients develop thrombotic problems initially but frequently terminate in leukemia, regardless of whether or not they've been treated with ³²P, which used to be a standard form of therapy.

Dr. S. S. Epstein (Case Western Reserve): I'd just like to say first of all how much I enjoyed your brilliant, stimulating presentation. I'm just wondering about this idea of yours of monitoring on a state level. On the basis of the figures you gave, say, 1000 deaths from leukemia per month in this country, there are roughly 20 leukemia deaths per month per state, of which half are myeloid, so you have 10 myeloid deaths per state per month. A 10% increase would be one death per state. Do you think this is of sufficient sensitivity to merit the kind of procedures you are recommending?

Dr. Lewis: I was thinking of it in terms of an increase of approximately 60 acute deaths per month in the entire U.S.

Dr. C. Ramel (Univ. of Stockholm): Another class which has a rather short lag period after exposure and which may open up some possibilities is nerve cancers in children, which I understand in Sweden has had a significant increase in the last decade.

Dr. Epstein: There was a paper by Jack Schubert of which I'm sure many of you are aware, reporting almost a tripling of the incidence of CNS tumors in children in Sweden from the years 1948 to about 1969. Many of us were fascinated by these data and presented it to the NCI for computer analysis. The incidence of CNS tumors was increased in children under the ages of 7 or 8. There was no excess of CNS tumors in people over that age. The curve has been found to be exactly flat for the American data. There is absolutely no increase in this country whatsoever. The reason why so many of us were interested in this is because in recent years or so there have been numerous reports on transplacental carcinogenesis, particularly to *N*-nitroso compounds. In a wide range of rodents that were given *N*-nitroso compounds, particularly in the latter part of pregnancy, there was a very high incidence of CNS tumors in the offspring. The question is then whether increase in incidence of tumors in Sweden reflects

prenatal exposure to *N*-nitroso compounds, and, if indeed this is the case in Sweden, why these data are not reflected in the American series.

Dr. G. Wolff (NCTR): I want to come back to the question that Dr. Kimball raised regarding the assumption of Dr. Sutton's statement about lack of repair leading to increased mutagenesis in embryos. To go one step beyond that, I think we should be cautious in accepting the notion that you do get increased mutagenesis when there is lack of repair. It might have heuristic value to consider this but we should keep in mind that there are cases where this may not be so. We've done experiments in which we find no excision repair in plants (Bill Hanawalt, Jim Trosko) and yet these plant cells don't seem to be throwing mutants all the time. And yesterday Dr. DeSerres told us about his work in *Neurospora* in which he had six repairless mutants and while two of them did indeed show an increase in mutations, some of them were mutation-resistant, and so the basic hypothesis might not be correct.

Dr. Cumming (ORNL): I want to get back to Dr. Sutton's initial comment on the increased sensitivity *in utero* and refer to the specific locus data. Paul Selby just completed a series of studies with Bill Russell on age-dependent differences in specific locus mutation rate. The mutation rates induced by x-rays on the first day postnatally, (first 9 hr after birth) are significantly below mutation rates for adults and not significantly different from the meagre *in utero* data. It appears that the very young organisms are less sensitive rather than more sensitive to mutation induction by x-rays, and the shift from the low mutation-induction rate to the high one comes within the first week of life (postnatal) in mice. So to assume that sensitivity to a mutagen is going to parallel what we will find in leukemia is a big jump. I think we have to really keep in mind that we're really concerned with mutation induction.

Dr. S. Abrahamson (Univ. of Wisconsin): That's the experiment in which newborn females were irradiated. They had 1/6 the mutation rate, and newborn males irradiated had 1/2 the mutation rate.

Dr. Cumming: That's right—in both cases, the mutation rates were significantly below the adult rate.

Dr. Abrahamson: But were there *in utero* experiments done by Shelby?

Dr. Cumming: Shelby didn't do them, there have been limited *in utero* experiments done by others, and those data do not differ statistically from Shelby's results for day. They're lower than the results from day one.

Dr. Lewis: I might say that the maternal and paternal exposures prior to conception were looked at carefully in a number of studies and don't show really significant associations in a consistent way.

There is something peculiar about the mongol result, by the way. If the Polani group is correct, the risk of mongolism is increased by an exposure that

is only 1-2 rad. One would not expect from among the existing estimates that there could be such a high rate of nondisjunction. What the explanation is, I have no idea. I suspect it might be related to the presence of some highly sensitive cells that are usually killed by 100 rad, but often affected by 1 rad. Now that's not mutation, of course, but rather nondisjunction.

Dr. Abrahamson: The Uchida data that you referred to earlier also showed this increase, but the increase was only in that group of females who were over the age of 38 at the time of irradiation.

Dr. Lewis: That was a very small sample. I think anything could come from that small sample.